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Transcriptional Induction of *MKP-1* in Response to Stress Is Associated with Histone H3 Phosphorylation-Acetylation

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Mitogen-activated protein (MAP) kinase phosphatase 1 (MKP-1) has been shown to play a critical role in mediating the feedback control of MAP kinase cascades in a variety of cellular processes, including proliferation and stress responsiveness. Although *MKP-1* expression is induced by a broad array of extracellular stimuli, the mechanisms mediating its induction remain poorly understood. Here we show that *MKP-1* mRNA was potently induced by arsenite and ultraviolet light and modestly increased by heat shock and hydrogen peroxide. Interestingly, arsenite also dramatically induces phosphorylation-acetylation of histone H3 at a global level which precedes the induction of *MKP-1* mRNA. The transcriptional induction of *MKP-1*, histone H3 modification, and elevation in *MKP-1* mRNA in response to arsenite are all partially prevented by the p38 MAP kinase inhibitor SB203580, suggesting that the p38 pathway is involved in these processes. Finally, analysis of the DNA brought down by chromatin immunoprecipitation (ChIP) reveals that arsenite induces phosphorylation-acetylation of histone H3 associated with the *MKP-1* gene and enhances binding of RNA polymerase II to *MKP-1* chromatin. ChIP assays following exposure to other stress agents reveal various degrees of histone H3 modification at the *MKP-1* chromatin. The differential contribution of p38 and ERK MAP kinases in mediating *MKP-1* induction by different stress agents further illustrates the complexity and versatility of stress-induced *MKP-1* expression. Our results strongly suggest that chromatin remodeling after stress contributes to the transcriptional induction of *MKP-1*.

The mitogen-activated protein (MAP) kinases play a central role in orchestrating many short- and long-term changes in the cell in response to extracellular stimuli (50). To date, three major MAP kinase subfamilies have been well characterized in mammalian cells: the extracellular signal-regulated kinase (ERK), the c-Jun N-terminal kinases/stress-activated protein kinase (JNK/SAPK), and p38 (11, 40, 50). Thus far, numerous proteins with a wide spectrum of biological functions have been identified as the targets of MAP kinase cascades, including protein kinases, cytoskeletal components, phospholipase A2, stalhmin, and the Na^+/H^+ antipump NHE1 (6, 23, 42). In addition to the proteins that function on the membrane or in the cytoplasm, MAP kinases also play a crucial role in regulating gene transcription. Upon activation, MAP kinases translocate from the cytoplasm to the nucleus, where they phosphorylate and activate a multitude of transcription factors, including c-Myc, c-Jun, c-Fos, Elk-1, and ATF-2, ultimately resulting in enhanced gene transcription (8, 24, 58). The fact that a broad variety of extracellular signals conscript MAP kinase cascades to convey their specific messages suggests that MAP kinase cascades serve a myriad of purposes and the cascades need to be tightly controlled.

The activities of all MAP kinases are regulated through reversible phosphorylation of two different amino acid residues (threonine and tyrosine) in the Thr-Xaa-Tyr signature motifs in their kinase subdomain VIII, where Xaa represents a characteristic of each MAP kinase subfamily (11, 40, 50). Activation of MAP kinases is catalyzed by their cognate dual-specificity MAP kinase kinases, whereas inactivation is primarily achieved by a group of MAP kinase phosphatases (MKPs) (9, 34). So far, 10 MKP family members have been cloned, and some of them are encoded by immediate-early genes, such as *MKP-1*, *MKP-2*, *PAC-1*, and *B23* (1, 35, 51, 55, 60). Since these proteins are localized in the nucleus and their expression can be induced by conditions that also activate MAP kinases, they are believed to play an important role in the attenuation of MAP kinase-mediated gene transcription (55, 60).

MKP-1, also referred to as 3CH134, CL100, or ERP, is the archetype of the MKP family (14, 35, 46, 55). In a number of systems, it has been demonstrated that the induction of *MKP-1* is concomitant with the inactivation of MAP kinases (43, 49, 55). Both in vitro and in vivo MKP-1 is able to interact with and effectively inhibit members of all three MAP kinase subfamilies (8, 32, 53, 55). Recent studies have demonstrated that MKP-1 protein can be stabilized by ERK-mediated phosphorylation (7) and that the catalytic activity of MKP-1 can also be stimulated through substrate binding (32, 53). These findings indicate that the activity of MKP-1 is controlled through multiple mechanisms involving both transcriptional and posttranslational regulation.

Although the induction of *MKP-1* expression by a broad variety of extracellular stimuli has been well documented (14, 34, 35, 39, 43, 46, 63), the underlying mechanisms regulating

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MKP-1 expression remain unclear. In this report, we studied the mechanisms involved in *MKP-1* induction by a number of stresses, including ultraviolet light (UVC), H_2O_2, heat shock, and the tumor promoter arsenite. We found that *MKP-1* mRNA is potently induced by arsenite and UVC and modestly increased by heat shock and H_2O_2. Arsenite and to a lesser extent UVC, also stimulated the activity of a 3-kbp *MKP-1* promoter. We demonstrated that *MKP-1* induction by arsenite and UVC was primarily mediated through the p38 MAP kinase cascade while the ERK pathway played a predominant role in mediating *MKP-1* induction by heat shock and H_2O_2. We further found that arsenite induced the phosphorylation and acetylation of histone H3 which preceded *MKP-1* induction. Treatment with the deacetylase inhibitor trichostatin A (TSA) increased basal *MKP-1* expression and augmented arsenite-induced *MKP-1* transcription. Finally, we demonstrated that arsenite induced the phosphorylation-acetylation of histone H3 on the *MKP-1* chromatin and increased the association of RNA polymerase II with the *MKP-1* gene. Enhanced histone H3 phosphorylation-acetylation of the *MKP-1* chromatin is also observed in cells treated with UVC and H_2O_2. Our results suggest that chromatin remodeling after stress may play an important role in *MKP-1* transcriptional activation.

MATERIALS AND METHODS

Cell culture, transfection, and treatments. Mouse C3H 10T1/2 cells (American Type Culture Collection) were cultured in minimum essential medium (Life Technologies, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, Utah). Transfection of C3H 10T1/2 cells was performed using Fugene 6 reagent (Roche Molecular Biochemicals, Indianapolis, Ind.) according to the manufacturer's specifications. Subconfluent cultures were rendered quiescent by serum starvation in minimal essential medium containing 0.5% FBS for 36 h prior to treatment. Treatment of the cells with UVC was performed as previously described (43). Heat shock treatment was carried out by switching to medium that was preheated to 42°C and incubating the cells in a 42°C chamber. Arsenite, H_2O_2, and TSA were directly added to the culture medium. U0126 (Promega, Madison, Wis.) and SB203580 (Sigma, St. Louis, Mo.), dissolved in dimethyl sulfoxide, were added to the medium to a final concentration of 10 μM 15 min prior to treatments.

***MKP-1* promoter–luciferase reporter assays.** To generate the *MKP-1*–Luc reporter construct (see Fig. 1B), a 3.2-kbp *Nco*I fragment from human *MKP-1* genomic DNA was cloned into the luciferase reporter vector pGL3-Enhancer (Promega) using standard techniques. This *MKP-1* genomic fragment spans positions −2975 to +247 (where position +1 refers to the transcription initiation site) of the human *MKP-1* gene (27). The *MKP-1*–Luc reporter construct together with pCH110 (SV40-lacZ) (Promega) were transiently transfected into C3H 10T1/2 cells. To establish cells stably carrying the *MKP-1*–Luc reporter, the reporter together with pcDNA3 (Invitrogen, Carlsbad, Calif.) was transfected into C3H 10T1/2 cells. After transfection, cells were selected in medium containing 500 μg of G418 per ml for 2 weeks. The resulting stable clones were pooled and maintained in medium containing 150 μg of G418 per ml. After arsenite or H_2O_2 treatment, the medium was removed and replaced with fresh medium containing 0.5% FBS. Six hours later, cells were harvested and luciferase activity was measured as previously described (43). β-Galactosidase activity was measured with a Galacto-Light kit (Tropix, Applied Biosystems, Bedford, Mass.) after the cell extract was heated at 48°C for 1 h to denature the endogenous enzyme. Luciferase activity was normalized to β-galactosidase values in transient-transfection assays and expressed in relative light units.

Western blot analysis and indirect immunofluorescence. To detect the modification of histone H3, crude histone proteins were extracted using sulfuric acid according to the procedures described by Cheung et al. (18). Samples were resolved on 12% NuPAGE gels (Invitrogen) using MES (morpholineethanesulfonic acid) buffer. To analyze the phosphorylation status of p38 or p44/p42 ERK, whole-cell lysates containing 20 μg of protein were resolved on a 10% NuPAGE gel using MOPS (morpholinepropanesulfonic acid) buffer. Immunoblotting was performed as described previously (16). Rabbit polyclonal antibodies against phospho-p38, total p38, phospho-p44/p42 ERK MAP kinases, phospho-histone H3 (Ser-10), and total histone H3 were purchased from Cell Signaling (Beverly, Mass.). Rabbit polyclonal antibodies recognizing phosphoacetyl-histone H3 (Ser-10, Lys-14) and acetyl-histone H3 (Lys-14) were purchased from Upstate Biotechnology (Lake Placid, N.Y.). Mouse monoclonal antibody against p44/p42 ERK was purchased from Transduction Laboratories (Lexington, Ky.).

In the immunofluorescence studies, Alexa 488-conjugated goat anti-rabbit and Alexa 568-conjugated goat anti-mouse secondary antibodies (Molecular Probes, Eugene, Oreg.) were used according to the manufacturer's protocols. Phosphohistone H3 was detected using a rabbit polyclonal antibody specific to Ser-10 phosphorylated histone H3 (Cell Signaling). Total histone H3 was detected using a mouse monoclonal antibody recognizing both phosphorylated and unphosphorylated histone H3 (a generous gift from William M. James, Intergen Discovery Products, Gaithersburg, Md.) (52). DAPI (4′,6′-diamidino-2-phenylindole) staining was performed as previously described (15). Samples were visualized by either fluorescence microscopy (Carl Zeiss, Thornwood, N.Y.) or confocal microscopy (Zeiss LSM-410 inverted confocal microscope equipped with a 63× NA 1.4 oil immersion objective). The confocal pinhole was set to obtain a spatial resolution of 0.4 μm in the horizontal plane and 1 μm in the axial dimension. Image processing and presentation were done using MataMorph 4.6.3 software (Universal Imaging, Inc., West Chester, Pa.).

MSK1 activity assay. The activity of MSK1 in cell lysates was assayed essentially as previously described by Deak et al. (25). Briefly, MSK1 was first immunoprecipitated from the cell lysates (1.6 mg of protein per sample) using a sheep polyclonal antibody (Upstate Biotechnology), extensively washed, and incubated at 30°C for 30 min in a 50-μl reaction volume containing 50 mM Tris-HCl (pH 7.5), 0.1 mM EGTA, 13 mM β-mercaptoethanol, 10 mM $MgCl_2$, 10 μM protein kinase A inhibitor (Sigma), 1 μM microcystin-LR, 20 μM ATP, 10 μCi of [γ-^{32}P]ATP, and 30 μM Crosstide (GRPRTSSFAEG) (Upstate Biotechnology). After termination of the kinase reaction, 10 μl of the reaction solution was loaded onto a SpinZyme phosphocellulose unit (Pierce, Rockford, Ill.). The phosphocellulose unit was washed four times with 150 mM phosphoric acid, and incorporation of ^{32}P into the peptide was determined by liquid scintillation counting.

Northern blot analysis. Total RNA was isolated with STAT-60 (Tel-Test B, Friendswood, Tex.). Northern blot analysis was performed as described previously (43). *MKP-1* and c-*fos* mRNAs were detected using mouse *MKP-1* and rat c-*fos* cDNA, respectively, as probes (21, 55). The mRNAs encoding β-actin and β-globin were detected using cDNA fragments from mouse EST clones (AA726293 and AI596092, respectively) as probes.

ChIP and PCR analysis. Chromatin was cross-linked using formaldehyde, broken down to an average size of ~2 kbp through brief sonication as described by Clayton et al. (20), and dissolved in radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris-HCl [pH 8.0], 1% Triton X-100, 0.1% sodium dodecyl sulfate, 0.1% sodium deoxycholate, 1 mM EDTA, 0.5 mM EGTA, 140 mM NaCl, 10 mM sodium butyrate, 20 mM β-glycerophosphate, 100 μM sodium orthovanadate, 2 μM leupeptin, 2 μM pepstatin A, and 1 mM phenylmethylsulfonyl fluoride [PMSF]). The resulting chromatin solution was divided equally for isolating input DNA and performing chromatin immunoprecipitation (ChIP) assays. Chromatin solutions were first incubated for 2 h at 4°C with 10 μl of rabbit preimmune serum 10 μl of purified antibody either specifically against phospho-histone H3 (Ser-10) (Cell Signaling) or specifically recognizing phosphoacetyl-histone H3 (Ser-10, Lys-14) (Upstate Biotechnology), or 10 μl of antiserum against RNA polymerase II (a generous gift from Michael Dahmus). Then bovine serum albumin (final concentration, 200 μg/ml), sonicated λ phage DNA (5 μg), and protein A-Sepharose beads (Pharmacia, Piscataway, N.J.) were added to the solutions and incubated overnight with gentle rotation at 4°C. The beads were washed three times with RIPA buffer, three times with RIPA buffer containing 500 mM NaCl, three times with washing buffer (10 mM Tris-HCl [pH 8.0], 0.25 M LiCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 20 mM β-glycerophosphate, 10 mM sodium butyrate, 100 μM sodium orthovanadate, 2 μM leupeptin, 2 μM pepstatin A, and 1 mM PMSF), three times with buffer A (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10 mM sodium butyrate, 20 mM β-glycerophosphate, 2 μM leupeptin, 2 μM pepstatin A, and 1 mM PMSF), and twice with buffer B (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10 mM sodium butyrate, 20 mM β-glycerophosphate). The beads were first treated with RNase A (50 μg/ml) for 1 h at 37°C and then with proteinase K (100 μg/ml) overnight. The input and immunoprecipitated chromatins were incubated at 65°C for ≥6 h to reverse the formaldehyde cross-links. The DNA was extracted with pheno-chloroform, precipitated with ethanol, and dissolved in water.

A pair of primers (TCAGCGGGGAGTTTTTGTG and CTGTGAGTGACC CTCAAAGTGG) was synthesized according to the sequence of the mouse *MKP-1* gene. With these primers, a 229-bp fragment that covers part of the second intron and third exon of the mouse *MKP-1* gene (46) can be amplified by PCR using mouse genomic DNA as a template. The primer pair CACGGCCG

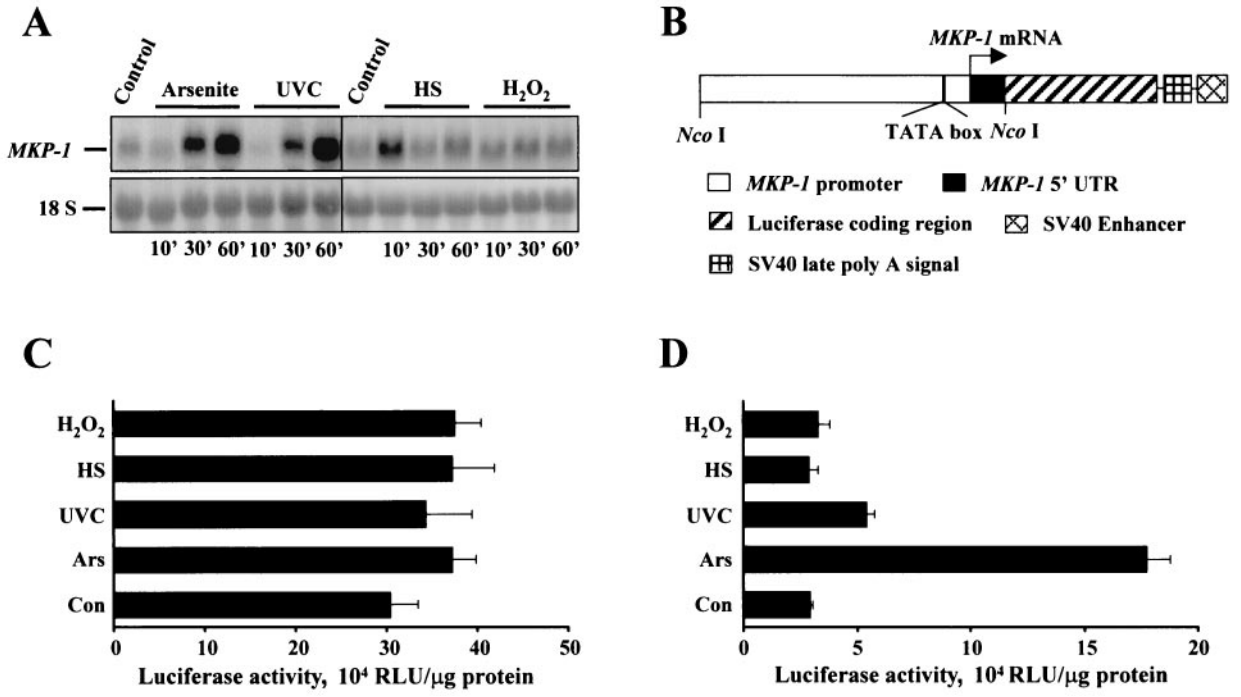

FIG. 1. Induction of *MKP-1* expression in C3H 10T1/2 cells by different stresses. (A) Time course of *MKP-1* mRNA induction by arsenite (400 μM), UVC (20 J/m^2), heat shock (42°C), and H_2O_2 (400 μM). *MKP-1* mRNA expression was assessed by Northern blotting using total RNA. RNA loading was indicated by 18S rRNA. (B) Diagram of the *MKP-1*–Luc reporter. UTR, untranslated region; SV40, simian virus 40. (C) Activation of the *MKP-1*–Luc reporter in response to different treatments in C3H 10T1/2 cells transiently transfected with 10 μg of the luciferase reporter together with 2 μg of pCH110 (SV40-lacZ). Luciferase activity was normalized to β-galactosidase measurements and expressed in relative light units (RLU). (D) Activation of the luciferase reporter in transfected cells that stably expressed the *MKP-1*-Luc construct. For the luciferase assays, cells were either heat shocked at 42°C for 45 min, treated for 3 h with either 100 μM arsenite or 200 μM H_2O_2, or irradiated with UVC (10 J/m^2). Data in panels C and D are means plus standard errors of the means from three independent experiments.

GTCCCTGTTGTTC and GTCGCGGTTGGAGTAGTAGGCG was used to amplify a 287-bp fragment of the c-*fos* promoter (18) using PCR. The β-*actin* primer pair AACACCCCAGCCATGTACG and ATGTCACGCACGATTT CCC (254-bp product) and the β-*globin* primer pair CAGTGAGTGGCACAG CATCC and CAGTCAGGTGCACCATGA TGT (247-bp product) were used in PCRs to amplify the respective sequences. For quantification, primers were end labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase and purified according to standard procedures. PCR amplification was carried out using the following parameters: 5 min at 94°C followed by 29 to 34 cycles of denaturation (94°C for 30 s), annealing (for 30 s, temperature optimized for each primer pair), and extension (72°C for 30 s) and an additional 10-min extension period after the final cycle. PCR products were resolved on 10% polyacrylamide–Tris-borate-EDTA gels and quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

RESULTS

Induction of *MKP-1* in mouse embryo fibroblasts. *MKP-1* expression in C3H 10T1/2 mouse embryo fibroblasts following treatment with various stresses was investigated by Northern blotting. While basal *MKP-1* mRNA levels were very low, arsenite treatment resulted in a potent increase in *MKP-1* mRNA abundance. Exposure to short-wavelength UVC also resulted in a strong induction of *MKP-1* mRNA. Consistent with a previous report (35), *MKP-1* mRNA was induced by both heat shock and H_2O_2, although the magnitude of induction was lower than that seen following arsenite treatment (Fig. 1A). The induction of *MKP-1* mRNA by each of these treatments was strictly time dependent (Fig. 1A).

To examine whether *MKP-1* induction was mediated through enhanced transcription, a luciferase reporter construct was generated using a human *MKP-1* genomic DNA fragment spanning nucleotides −2975 to +247, where nucleotide +1 is the transcription start site (Fig. 1B). This reporter was transiently transfected into C3H 10T1/2 cells, and luciferase activity was assayed after treatment of cells with arsenite, H_2O_2, heat shock, or UVC. To our surprise, none of these treatments had an appreciable effect on *MKP-1*–Luc reporter activity in the transient-transfection assays (Fig. 1C). However, when the construct was stably integrated into C3H 10T1/2 cells, treatment of the pooled cells with 100 μM arsenite for 3 h resulted in a six-fold increase in *MKP-1* promoter activity (Fig. 1D). UVC irradiation also modestly enhanced *MKP-1*–Luc reporter activity. In contrast, neither heat shock nor H_2O_2 had a significant effect on the activity of the ectopic *MKP-1* promoter (Fig. 1D).

Both p38 and ERK are implicated in the transcriptional induction of *MKP-1*. To investigate the role of MAP kinase pathways in the transcriptional induction of *MKP-1*, the effects of the MEK1/2 inhibitor U0126 and the p38 inhibitor SB203580 on *MKP-1* expression were examined. Northern blot analysis indicated that SB203580 substantially inhibited *MKP-1* mRNA induction by arsenite, reducing the *MKP-1* mRNA level by more than 70%. In contrast, the MEK inhibitor U0126 had no significant effect on *MKP-1* mRNA induction by arsenite (Fig. 2A). Likewise, SB203580, but not U0126, significantly attenuated *MKP-1* mRNA induction by UVC (Fig. 2A). Inter-

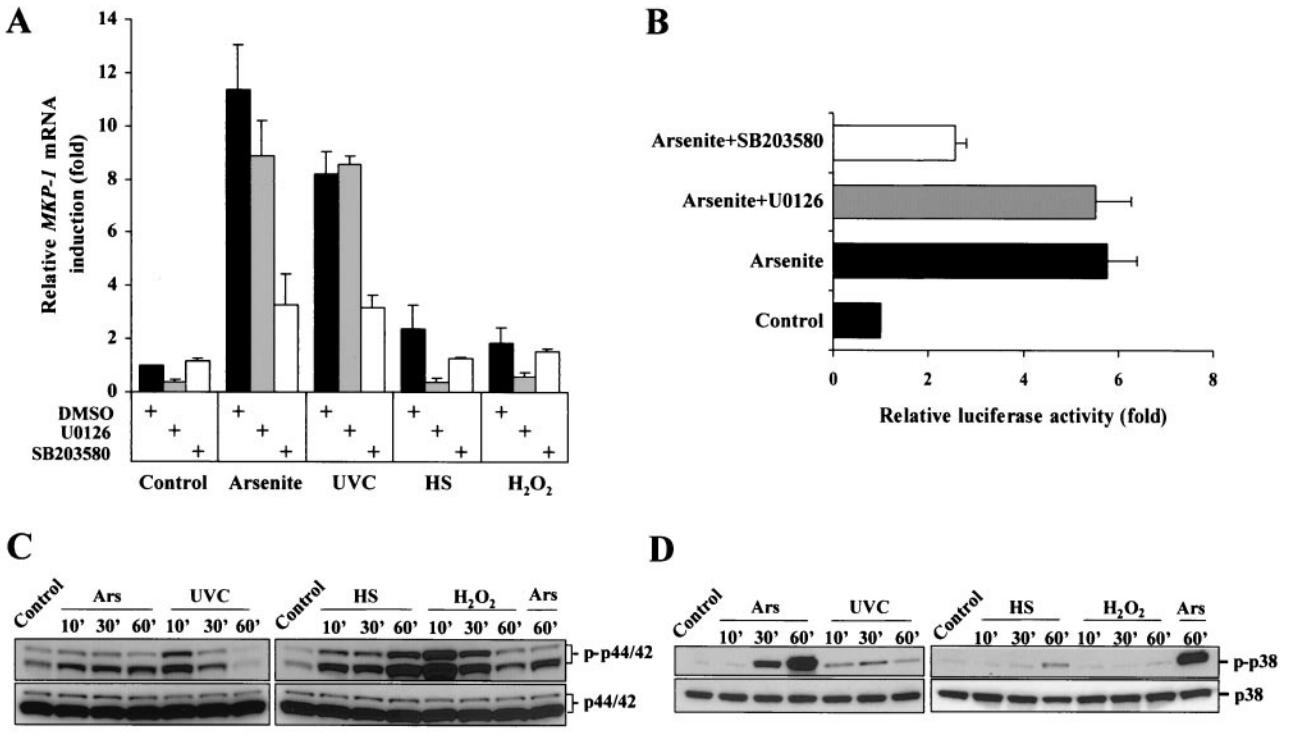

FIG. 2. Role of ERK and p38 in mediating *MKP-1* induction by stress in C3H 10T1/2 cells. (A) Northern blot analysis to monitor *MKP-1* mRNA levels in response to arsenite (400 μM), UVC (20 J/m^2), heat shock (42°C) (HS), or H_2O_2 (400 μM). U0126 (10 μM) or SB203580 (10 μM) was added to the medium 15 min before stimulation with the stress agents. Cells were harvested 1 h after exposure to stress. *MKP-1* mRNA signals were normalized to 18S rRNA signals and expressed as *MKP-1* mRNA induction relative to the levels in control cells. Values are means plus standard errors of the means from three independent experiments. DMSO, dimethyl sulfoxide. (B) Effects of U0126 and SB203580 on *MKP-1*–Luc reporter activation induced by arsenite (100 μM, 3 h). Data are means plus standard errors of the means from three independent experiments. (C) Kinetics of ERK activation. Western blotting was done with an antibody specific for phosphorylated ERK1/2 (upper panel) or an antibody recognizing total ERK (lower panel). (D) Kinetics of p38 activation. A Western blot using an antibody specific for phospho-p38 (upper panel) or an antibody for total p38 (lower panel) is shown. Cells were stimulated with arsenite (400 μM), UVC (20 J/m^2), heat shock (42°C), or H_2O_2 (400 μM) for the indicated times or left untreated.

estingly, U0126 significantly inhibited *MKP-1* induction by both heat shock and H_2O_2, while SB203580 had little inhibitory effect on *MKP-1* induction by these treatments (Fig. 2A). Further evidence that p38 regulates the transcriptional induction of *MKP-1* by arsenite was obtained in assays showing SB203580-mediated inhibition of the *MKP-1*–Luc reporter, with little effect by the MEK inhibitor U0126 (Fig. 2B).

To understand the molecular basis underlying *MKP-1* induction in response to stress, we examined the activation kinetics of both ERK and p38 following exposure to a variety of stress treatments. Activation of ERK and p38 was monitored by Western blot analysis using antibodies specific to the respective phosphorylated isoforms. While ERK MAP kinases were potently activated by H_2O_2 and heat shock, they were only modestly activated by UVC irradiation and weakly stimulated by arsenite (Fig. 2C). By contrast, arsenite substantially stimulated p38 phosphorylation, which increased with time. UVC irradiation also resulted in a significant increase in p38 phosphorylation. Unlike arsenite and UVC, heat shock was a poor activator of p38 at the 60-min time point while H_2O_2 did not affect p38 phosphorylation under the conditions of the assay (Fig. 2D). Western blotting to assess the levels of both phosphorylated and nonphosphorylated kinases revealed that protein loading was comparable among all samples (Fig. 2C and D). Taken together, these results suggest that both p38 and ERK contribute to the induction of *MKP-1* expression and the contributions of different MAP kinase subfamilies vary according to the kinetics and magnitude of their activation.

Differential phosphorylation and acetylation of histone H3 in response to stress stimulation correlate with MSK1 activity. Recent studies have provided abundant evidence to suggest that the phosphorylation-acetylation of histone H3 following mitogenic stimulation plays an important role in mediating transcriptional activation of immediate-early genes such as c-*fos* and c-*jun* (13, 18, 20, 56). Since *MKP-1* is also an immediate-early gene, we tested whether histone H3 phosphorylation-acetylation might be involved in *MKP-1* induction by arsenite. Following stimulation of C3H 10T1/2 with arsenite for various lengths of time, phosphorylated histone H3 was detected by indirect immunofluorescence using an antibody that specifically recognized phospho-histone H3 (Ser-10). Since epidermal growth factor (EGF) together with anisomycin has been shown to induce histone H3 phosphorylation and superinduction of immediate-early genes (20), this combination treatment was used as a positive control (Fig. 3A). Compared with control cells, where the level of phosphorylated histone H3 was low, arsenite triggered a rapid accumulation of phospho-histone H3 in the nucleus. The levels of phosphorylated histone

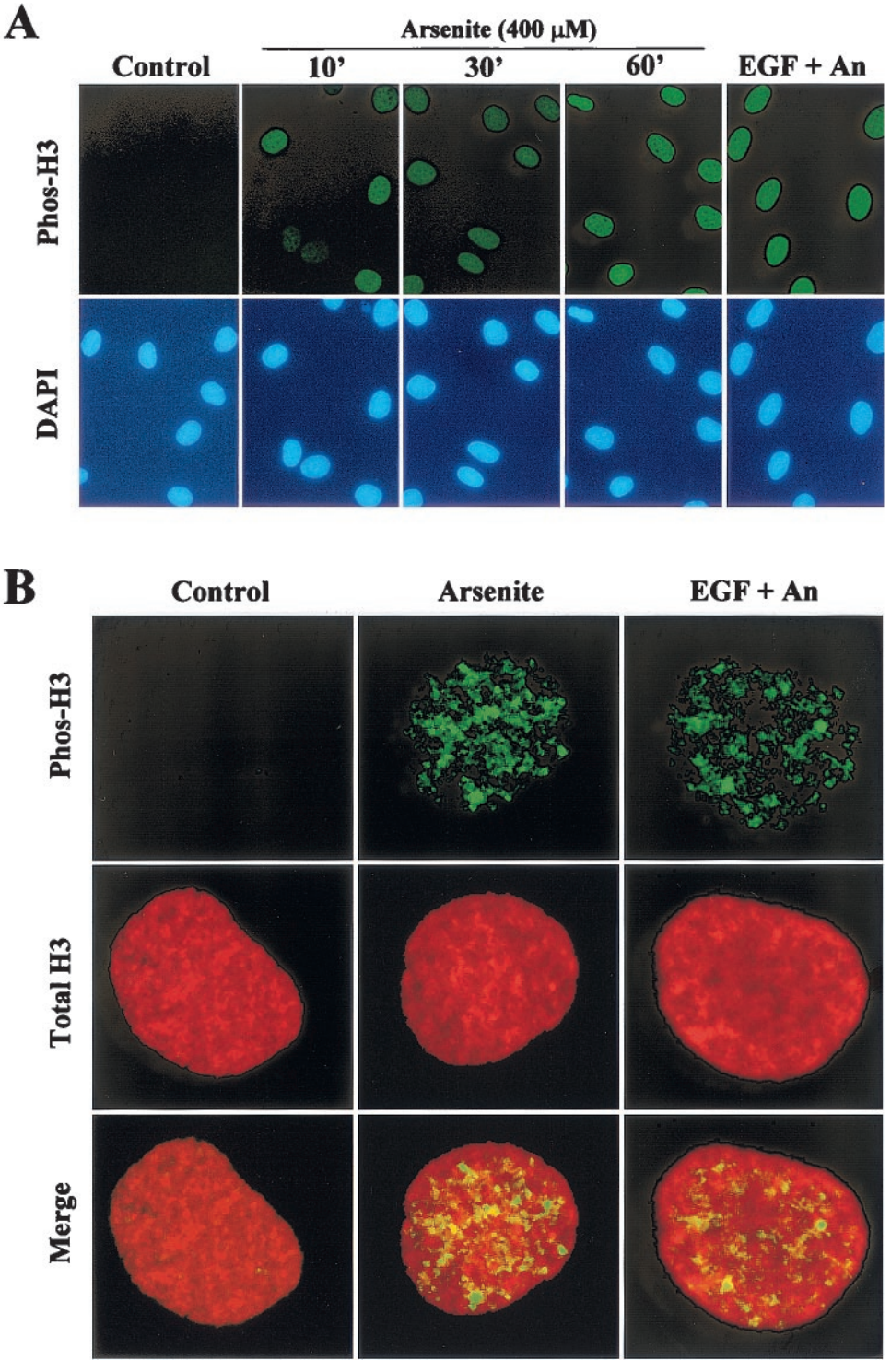

FIG. 3. Immunofluorescent detection of Ser-10-phosphorylated histone H3. C3H 10T1/2 cells were treated with arsenite (400 μM) for the indicated times. Histone H3 phosphorylation was detected by indirect immunofluorescence. (A) Time course of histone H3 phosphorylation in response to arsenite treatment. (Upper panels) Immunofluorescent detection of phosphohistone H3 (Ser-10) (Phos-H3); (lower panels) DAPI staining of cell nuclei. Cells treated with EGF (50 ng/ml) and anisomycin (10 μg/ml) (An) for 60 min were included as a positive control. (B) Confocal microscopy of histone H3 phosphorylation. Unstimulated cells (control), cells treated with arsenite (400 μM) for 60 min, or cells stimulated with EGF (50 ng/ml) together with anisomycin (10 μg/ml) for 60 min were first stained with a rabbit phospho-histone H3 antibody (green signal) and then stained with a mouse monoclonal antibody against total histone H3 (red signal). Representative fluorescent images are shown.

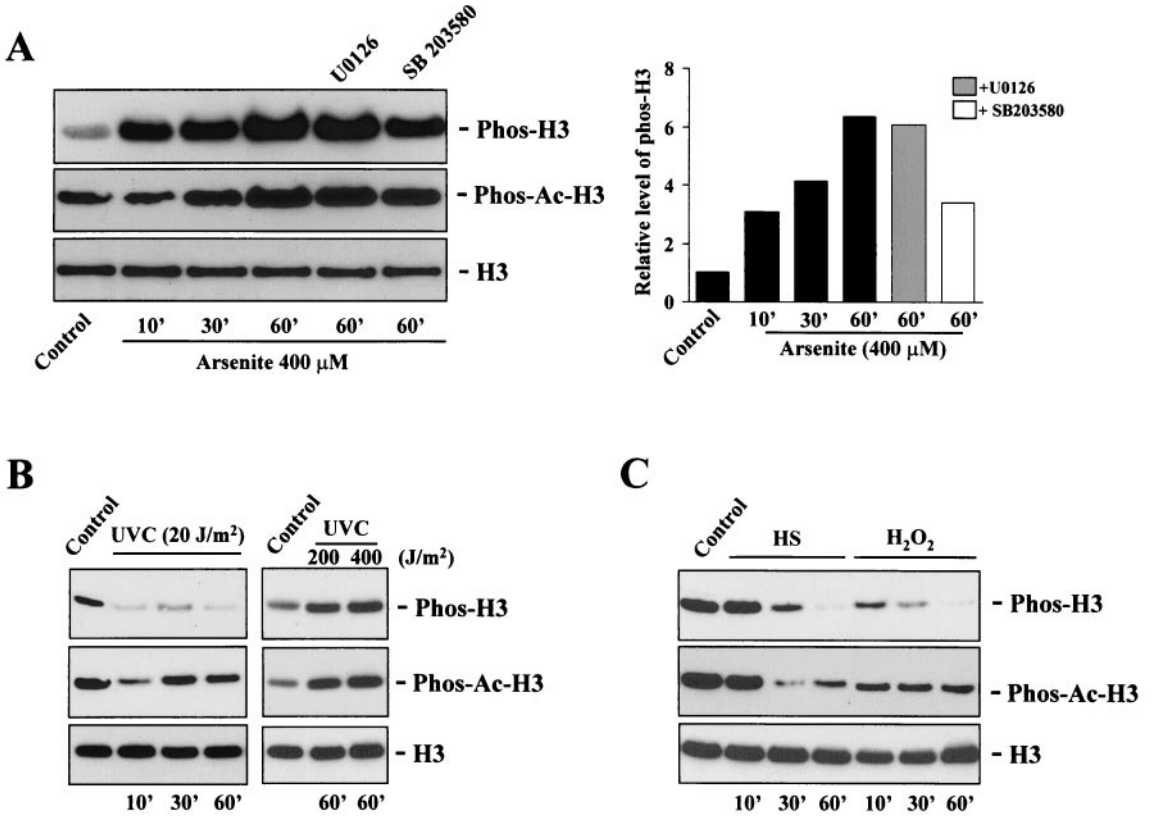

FIG. 4. Global levels of histone H3 phosphorylation-acetylation in cells stimulated with stress. C3H 10T1/2 cells were treated with arsenite (400 μM), UVC (20, 200, and 400 J/m^2), heat shock (42°C), or H_2O_2 (400 μM) for the indicated times in the absence or presence of U0126 or SB203580. Cells were lysed to extract crude histone proteins. Western blot analysis was performed on the crude histone samples using an antibody specific to phospho-histone H3 (Ser-10) (Phos-H3), phosphoacetylated histone H3 (Ser-10, Lys-14) (Phos-Ac-H3), or total histone H3. (A) Histone H3 phosphorylation-acetylation in cells treated with arsenite. The graph shows the relative levels of phospho-histone H3. (B) Histone H3 phosphorylation-acetylation in cells irradiated by UVC. (C) Histone H3 phosphorylation-acetylation in cells stimulated by heat shock (42°C) (HS) or H_2O_2 (400 μM).

H3 after exposure to arsenite for 60 min were comparable to those seen with the combination of EGF and anisomycin (Fig. 3A).

It has been reported that EGF-stimulated histone H3 phosphorylation is restricted to a small subset of nucleosomes that are associated with active gene transcription (4, 5, 17, 18, 20). Recently, it was found that in *Drosophila* salivary glands, heat shock significantly stimulated histone H3 phosphorylation at a few loci where the heat shock protein genes are located, while the global level of phosphorylated histone H3 decreased dramatically (47). To investigate whether arsenite stimulates phosphorylation of histone H3 throughout the entire nucleus or at a few loci, confocal microscopy was performed using a rabbit polyclonal antibody specifically recognizing Ser-10-phosphorylated histone H3. A mouse monoclonal antibody recognizing both phosphorylated and nonphosphorylated histone H3 was used to visualize all histone H3 proteins (Fig. 3B). In unstimulated cells, only a few loci were intensely stained by the phospho-histone H3 antibody (Fig. 3B). In cells stimulated with EGF and anisomycin, phospho-histone H3 was markedly more abundant and formed a punctate staining pattern in the nucleus (Fig. 3B). Like EGF and anisomycin, arsenite stimulation also resulted in a dramatic increase in phospho-histone H3, revealing a similar punctate staining pattern in the nucleus (Fig. 3B). The marked difference between the staining patterns of the phosphorylated histone H3 and those of the total histone H3 protein in arsenite-stimulated cells strongly suggests that arsenite induces the phosphorylation of a subset of histone H3 molecules (Fig. 3B).

To further examine the effect of various stresses on histone H3 modification, histone proteins were extracted with sulfuric acid from cells stimulated with arsenite, UVC, heat shock, and H_2O_2. Histone H3 modification was examined by Western blot analysis using antibodies specific for either phospho-histone H3 (Ser-10) or dually modified histone H3 (Ser-10, Lys-14). Arsenite stimulated a rapid phosphorylation of histone H3 in a time-dependent manner. This modification was visible within 10 min after the addition of arsenite to the medium and continued to increase over the time period studied (Fig. 4A). Phosphoacetyl-histone H3 increased in a time-dependent fashion with kinetics similar to that of histone H3 phosphorylation (Fig. 4A), supporting the notion that phosphorylation and acetylation of histone H3 are tightly coupled events (17). Interestingly, the p38 inhibitor SB203580 modestly reduced arsenite-triggered phosphorylation-acetylation of histone H3, while the MEK inhibitor U0126 had little effect (Fig. 4A).

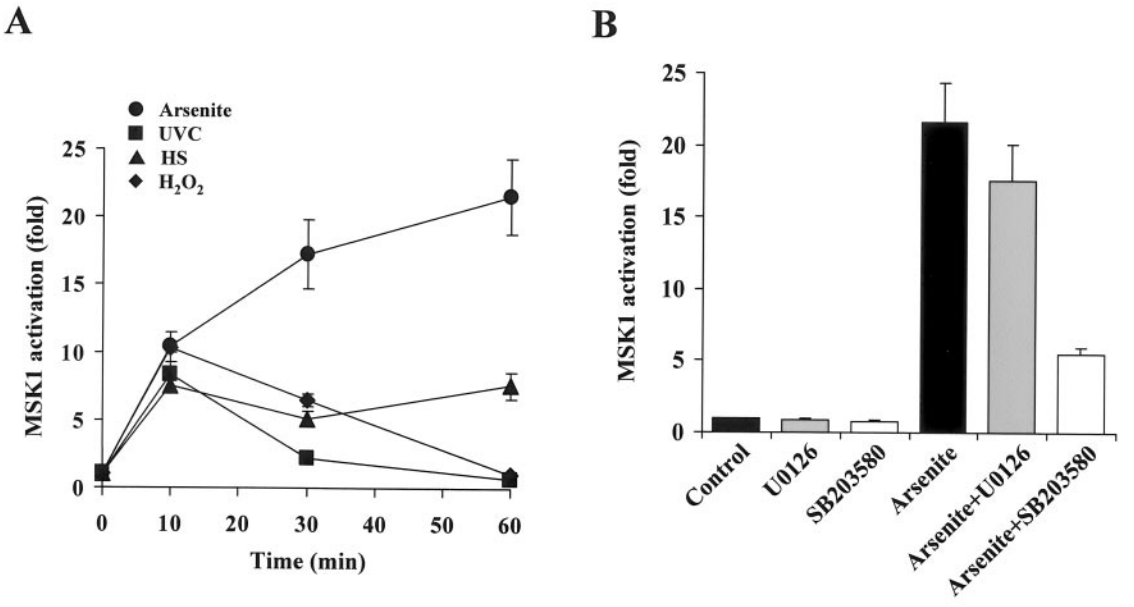

FIG. 5. Activation of MSK1 in response to stress. C3H 10T1/2 cells were treated for the times indicated. MSK1 activity in cell lysates was assayed using a Crosstide peptide as a substrate. (A) Kinetics of MSK1 activation in cells stimulated with arsenite (400 μM), UVC (20 J/m^2), heat shock (42°C) (HS), or H_2O_2 (400 μM). (B) Effect of U0126 or SB203580 on MSK1 activation triggered by arsenite. Cells were stimulated with arsenite (400 μM) for 60 min in the presence of either U0126 or SB203580 and harvested to assess MSK1 activity. The data are means plus standard errors of the means from three independent experiments, with each determination carried out in duplicate.

Unlike arsenite, which profoundly stimulated histone H3 phosphorylation and acetylation, exposure to 20 J of UVC per m^2 decreased the levels of both phosphorylated and phosphoacetylated histone H3 at a global level, while higher doses (200 and 400 J/m^2) of UVC irradiation significantly increased global histone H3 phosphorylation and acetylation (Fig. 4B). Interestingly, treatment of cells with both heat shock (42°C) and H_2O_2 (400 μM) significantly decreased global histone H3 modification (Fig. 4C).

MSK1 activation by stress agents. MSK1 is a protein kinase that can be activated by both mitogens and stress, and it lies downstream of both p38 and ERK (25). Recent studies suggest that MSK1 may play an important role in mediating the phosphorylation of histone H3, thus serving as a link between the MAP kinase cascades and the nucleosomal events (56). To examine whether MSK1 could be involved in histone H3 phosphorylation and thus be implicated in *MKP-1* induction by stress, we examined the kinetics of MSK1 activation following exposure to stressful treatments. MSK1 was immunoprecipitated and assayed using a synthetic peptide (Crosstide) as a substrate (25) and was found to be significantly activated by all four stresses. Arsenite was found to be the best inducer, eliciting a >10-fold increase in MSK1 activity within 10 min and a sustained >20-fold increase after 1 h of arsenite exposure (Fig. 5A). Although other treatments, including UVC, heat shock, and H_2O_2, also significantly activated MSK1, this elevated activity was transient and decreased soon thereafter (Fig. 5A). Consistent with previous reports that p38 plays a dominant role in mediating MSK1 activation by arsenite (25), SB203580 abolished MSK1 activation by arsenite while U0126 had little effect on its activation (Fig. 5B). The overall similar kinetics between histone H3 phosphorylation and MSK1 activation and the similar susceptibility profiles for the two MAP kinase inhibitors strongly support the notion that MSK1 may play a significant role in modulating histone H3 modifications in response to stress.

Histone deacetylase inhibitor TSA augments *MKP-1* induction. To assess the influence of histone modification on *MKP-1* induction, the effect of the deacetylase inhibitor TSA (20) on *MKP-1* expression was examined. Indeed, pretreatment of cells with TSA augmented histone H3 acetylation on Lys-14, as revealed by Western blotting (Fig. 6A). On Northern blots, TSA alone was found to significantly enhance basal *MKP-1* mRNA levels and to substantially augment *MKP-1* mRNA induction by arsenite (Fig. 6B). The effect of TSA on *MKP-1* promoter activity, examined using cells stably carrying the *MKP-1*–Luc reporter, revealed results similar to those seen by Northern blot analysis: the *MKP-1* promoter activity was three- to six-fold higher in TSA-treated cells and was further induced by the addition of arsenite (Fig. 6C).

Transcriptional induction of *MKP-1* is associated with phosphorylation and acetylation of histone H3 on the *MKP-1* chromatin. To examine whether arsenite stimulates phosphorylation of histone H3 proteins associated with the *MKP-1* gene, control and arsenite-stimulated cells were treated with formaldehyde to cross-link chromatin, chromatin-associated proteins, and genomic DNA. Following solubilization with sodium dodecyl sulfate and sonication, ChIP assays were carried out using antibodies specifically recognizing either phospho-histone H3, phosphoacetyl-histone H3, or RNA polymerase II. Genomic DNA present in the immunoprecipitates was extracted and analyzed by PCR using ^{32}P-labeled primers derived from the mouse genes. The specificity and accuracy of these assays were monitored by performing mock ChIP reactions in

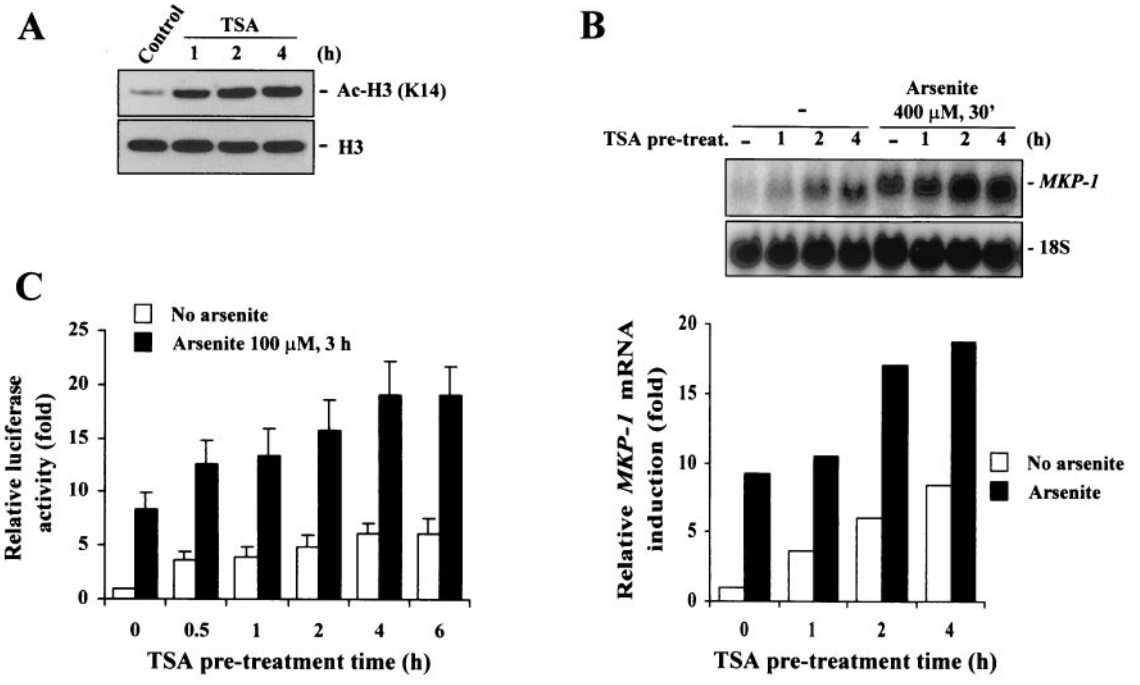

FIG. 6. Effect of TSA on *MKP-1* expression. (A) Histone H3 acetylation in cells treated with TSA. C3H 10T1/2 cells were treated with 500 ng of TSA per ml for the indicated times and harvested to extract crude histone proteins. Western blotting was performed using an antibody specifically recognizing acetyl-histone H3 (Lys-14) (Ac-H3). (B) Northern blot analysis of *MKP-1* mRNA. C3H 10T1/2 cells were either pretreated with TSA (500 ng/ml) for the times indicated or received no pretreatment. Cells were either left unstimulated or further stimulated with arsenite (400 μM) for 30 min. The relative mRNA induction is quantitatively shown in the graph. Cells pretreated with TSA for 0 h refers to cells that did not receive TSA pretreatment. (C) Effect of TSA on *MKP-1*–Luc reporter activity in unstimulated and arsenite-stimulated C3H 10T1/2 cells. C3H 10T1/2 cells carrying the *MKP-1*–Luc reporter were either pretreated with 500 ng of TSA per ml for the indicated times or left untreated. Cells were then stimulated with 100 μM arsenite for 3 h. Six hours later, luciferase activity was assayed and normalized to protein amount. Data are relative to the basal luciferase activity: luciferase activity in treated cells/luciferase activity in untreated cells.

the absence of antibody, carrying out PCR assays in the linear range of amplification, and executing PCR amplifications using DNA from the input chromatins as templates (Fig. 7A, C, and D). ChIP assays showed an approximate 3.2-fold increase in the levels of phosphorylated histone H3 on the *MKP-1* chromatin following exposure to arsenite (Fig. 7A). Interestingly, SB203580 substantially inhibited histone H3 phosphorylation at the *MKP-1* chromatin. The association between histone H3 phosphorylation and *MKP-1* transcription was demonstrated by ChIP assays using an antibody against RNA polymerase II. RNA polymerase II associated with the *MKP-1* gene was significantly increased after arsenite treatment but was considerably inhibited by pretreatment with SB203580 (Fig. 7A). However, neither treatment had appreciable effects on either the amount of phospho-histone H3 or the amount of RNA polymerase II associated with the chromatin of the housekeeping gene β-*actin* (Fig. 7A). In addition, treatment of cells with arsenite did not induce histone H3 phosphorylation or RNA polymerase II association at the chromatin of a transcriptionally inactive gene β-*globin* (Fig. 7A). These results suggest that arsenite induces nucleosomal changes and activates transcription at only a subset of genes. This notion was supported by Northern blot analysis. Arsenite treatment did not significantly change the β-*actin* mRNA levels, even though it potently induced the transcription of both *MKP-1* and c-*fos* (Fig. 7B). No β-*globin* mRNA was detected in C3H 10T1/2 cells under any treatment conditions, while a strong signal was detected in the positive control RNA (differentiated erythroleukemic cells) (Fig. 7B).

ChIP assays were also performed using an antibody specific to dually modified histone H3 (phosphorylated Ser-10, acetylated Lys-14) at the *MKP-1* and c-*fos* loci. Arsenite significantly increased phosphoacetylation of histone H3 at the *MKP-1* chromatin, a process that was notably inhibited by SB203580 (Fig. 7C). Arsenite treatment also enhanced histone H3 phosphorylation-acetylation at the c-*fos* chromatin. However, SB203580 had little effect on this process (Fig. 7C), an observation that is consistent with Northern blotting results showing a more prominent role for ERK than for p38 in arsenite-mediated c-*fos* induction (Fig. 7B). Examination of histone H3 modification at the loci of *MKP-1* and c-*fos* in cells treated by UVC or H_2O_2 revealed that phosphorylation-acetylation was enhanced at these loci, although the change triggered by H_2O_2 was less prominent (Fig. 7D). Taken together, our results suggest that nucleosomal changes mediated by histone H3 modification play an important role in mediating the transcriptional induction of *MKP-1* by stress stimuli.

DISCUSSION

Histone H3 modification and activation of *MKP-1* transcription. *MKP-1* can be induced by a myriad of extracellular stim-

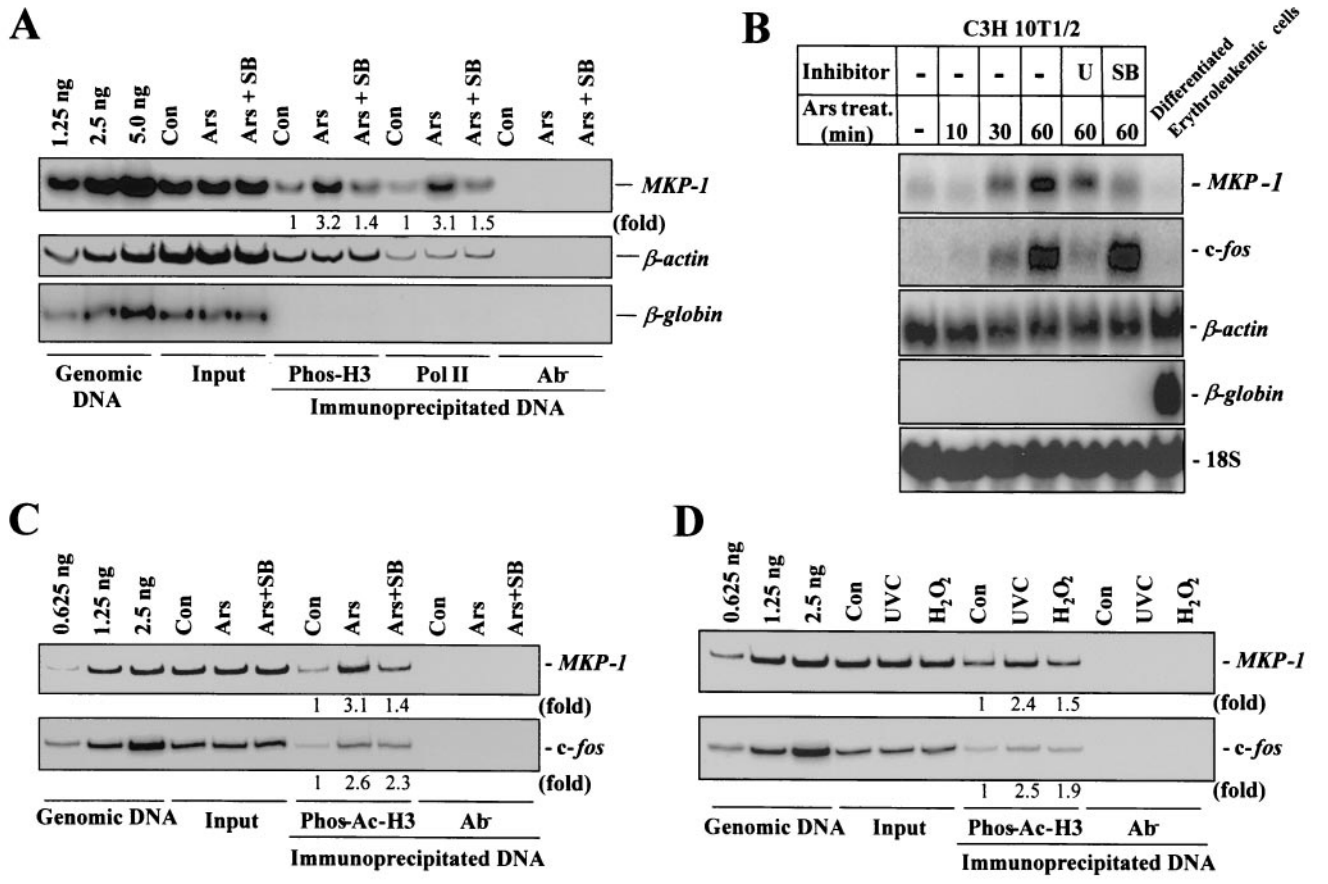

FIG. 7. Phosphorylation-acetylation of histone H3 on the *MKP-1* chromatin. (A) Phosphorylation of histone H3 (Ser-10) and association of RNA polymerase II (Pol II) with *MKP-1* chromatin in control and arsenite-treated cells. C3H 10T1/2 cells were treated with 400 μM arsenite for 60 min in the presence or absence of SB203580. ChIP assays were performed using anti-phospho-H3 antibody, anti-Pol II antiserum, or preimmune serum (Ab^-). The DNA recovered from the antibody-bound fractions as well as the DNA from input chromatin (Input) were analyzed for the presence of *MKP-1*, β-*actin*, and β-*globin* sequences by PCR using ^{32}P-labeled primers. Results of representative experiments are shown. (B) Levels of *MKP-1*, c-*fos*, β-*actin*, and β-*globin* mRNAs in cells stimulated with arsenite (400 μM) (Ars). U0126 or SB203580 (each 10 μM) was added to the medium 15 min prior to the addition of arsenite. That equal amounts of RNA were loaded was assessed by blotting with 18S rRNA. RNA from differentiated erythroleukemic BB88 cells was used as a positive control for β-*globin* mRNA. (C) ChIP assays performed on arsenite-stimulated cells using anti-phosphoacetyl-histone H3 antibodies. The DNA recovered from the antibody-bound fractions, the DNA from input chromatin, and the genomic DNA were analyzed by hot PCR for *MKP-1* and c-*fos*. (D) ChIP assays performed using an antibody specific to phosphoacetylated histone H3 on cells stimulated by UVC (20 J/m^2, 60 min) and H_2O_2 (400 μM, 60 min). The DNA recovered was analyzed for *MKP-1* and c-*fos* sequences by PCR. The DNA recovered was analyzed for *MKP-1* and c-*fos* sequences by PCR. Numbers below the gels are intensities of the bands quantitated using a densitometer and expressed as the difference in band intensity in treated versus untreated cells.

uli, including growth factors and stresses (34, 46), but the mechanisms contributing to its induction remain unclear. In this study, we explored the mechanisms involved in *MKP-1* induction by a variety of stresses, including UVC, heat shock, H_2O_2, and arsenite. We showed that in C3H 10T1/2 cells *MKP-1* mRNA was strongly increased by exposure to arsenite and UVC and modestly induced by heat shock and H_2O_2. Using pharmacological inhibitors specific for the ERK and p38 cascades, *MKP-1* induction by arsenite and UVC was found to be predominantly mediated by the p38 pathway, while *MKP-1* induction by heat shock and H_2O_2 was primarily dependent on the ERK pathway (Fig. 2). These differential contributions of the two MAP kinase subfamilies to *MKP-1* expression can be explained by the differential activation of these kinases by each treatment. Arsenite potently activated p38 but only weakly stimulated ERK. UVC significantly activated p38 even though it also resulted in a transient activation of the ERK cascade. On the other hand, heat shock and H_2O_2 potently activated ERK, while they had little effect on p38 activity (Fig. 2).

An interesting finding from this study is that arsenite potently stimulates phosphorylation-acetylation of histone H3. Similar to combined treatment with EGF and anisomycin (4), arsenite did not increase histone H3 phosphorylation uniformly throughout the entire genome, but it did do so at a number of specific loci (Fig. 3B). By contrast, heat shock and H_2O_2 treatments actually decreased global histone H3 modification (Fig. 4). As for UVC, low doses of irradiation (20 J/m^2) decreased histone phosphorylation-acetylation, while high doses (200 and 400 J/m^2) significantly enhanced phosphorylation-acetylation of histone H3 (Fig. 4B), in agreement with a previous report (10). Since histone acetylation levels have long been believed to correlate with the transcription status of many

genes (17, 28, 38, 57), decreases in global histone H3 phosphorylation-acetylation may represent a cellular defense mechanism to halt the transcription of nonessential genes. As previously reported, exposure to heat stress as well as to moderate doses of oxidative stress represses the expression of many genes (2, 45). Very recently, Nowak and Corces demonstrated that heat shock of *Drosophila* salivary gland dramatically decreases global histone H3 phosphorylation while it substantially increases histone H3 phosphorylation at a few loci containing the genes encoding heat shock proteins (47).

Recent studies have demonstrated that in response to mitogenic stimuli, histone H3 phosphorylation-acetylation occurs preferentially at the c-*fos* promoter in an ERK-dependent manner (18, 20). In addition to c-*fos*, c-*jun*- and c-*myc*-associated nucleosomes have been reported to undergo preferential histone H3 phosphoacetylation upon gene activation (13, 20, 30). In the present study, we found that arsenite, as well as UVC and H_2O_2, also induced phosphorylation-acetylation of histone H3 at the c-*fos* promoter (Fig. 7C and D). That arsenite-triggered *MKP-1* transcriptional induction is mediated by histone modification is supported by the following findings: (i) histone H3 phosphorylation and acetylation preceded *MKP-1* mRNA induction (compare Fig. 1A with Fig. 4A); (ii) treatment with the deacetylase inhibitor TSA increased *MKP-1* basal transcription and also augmented arsenite-induced *MKP-1* expression (Fig. 6); (iii) SB203580 partially inhibited histone H3 phosphorylation-acetylation induced by arsenite and also compromised the transcriptional induction of *MKP-1* as indicated by the attenuated *MKP-1* mRNA increase and abolished *MKP-1*–Luc reporter activation (Fig. 2A and B and 4A); and (iv) arsenite-stimulated histone H3 phosphorylation and acetylation on the *MKP-1* chromatin correlated with the enhanced RNA polymerase II binding to the *MKP-1* gene (Fig. 7A and C), and both processes were inhibited by SB203580. It should be noted that the close association between *MKP-1* induction and histone modification observed in arsenite-treated cells is likely to be seen also with other stresses. UVC irradiation, and to a lesser extent H_2O_2, also enhanced histone H3 phosphorylation-acetylation at the *MKP-1* chromatin (Fig. 7D), despite the global reduction in phosphorylated-acetylated histone H3 following these treatments (Fig. 4B and C).

Although the strong correlations between histone H3 modification and transcriptional activation of *MKP-1* suggest that the two processes are mechanistically linked, the exact mechanisms involved in stress-triggered *MKP-1* transcription are still unclear. Histone modification triggered by stressful stimuli could influence *MKP-1* transcription through two possible mechanisms. The phosphoepitope on histone H3 may provide binding sites for the recruitment of coactivators such as p300/CBP or chromatin remodeling complexes, thus facilitating the assembly of active transcription complexes (17, 28, 44). Alternatively, phosphorylation may mediate changes in the nucleosome structure, thus increasing chromatin accessibility to transcription factors and ultimately leading to enhanced transcription (17, 22). The fact that the histone deacetylase inhibitor substantially induced *MKP-1* expression (Fig. 6) indicates that histone acetylation alone can enhance *MKP-1* transcription. Since *MKP-1* can be induced by a myriad of extracellular stimuli, it is possible that *MKP-1* induction by some treatments may involve only histone H3 acetylation.

An intriguing observation from this study is that a ~3-kbp *MKP-1* promoter is sufficient for its activation by some but not all treatments. We found that arsenite, and to a lesser extent UVC, but not heat shock or H_2O_2, significantly stimulated luciferase activity from this reporter (Fig. 1D). Similarly, neither serum nor a cyclic AMP (cAMP) analog could increase the *MKP-1*–Luc reporter activity (data not shown), even though both agents have been shown to potently increase *MKP-1* mRNA (14, 46). Preliminary studies in our laboratory indicate that the increase in *MKP-1* mRNA levels in response to extracellular stimuli such as serum, H_2O_2, and heat shock is primarily due to enhanced transcription rather than increased mRNA stability (data not shown). This is consistent with the report that induction of *MKP-1* expression by hypoxia involves transcriptional activation, not mRNA stabilization (41). Two possibilities could account for the lack of response of the *MKP-1*–Luc reporter to these treatments (H_2O_2, heat shock, serum, and cAMP). First, it is possible that the transcriptional activation of *MKP-1* by certain treatments, such as serum, cAMP, heat shock and H_2O_2, involves chromatin remodeling of a genomic segment larger than that used here, as described for β-*globin* (64). Secondly, transcriptional activation of *MKP-1* could be mediated through multiple *cis* elements via cooperation of distinct transcription factors. In many genes, transcriptional control elements have been identified in either far upstream or far downstream regions from the transcription initiation site or even in their introns (19, 29, 33). Although the 2,975-bp promoter used in our studies contains a number of consensus *cis*-element sequences (including two cAMP response elements, an AP-1-binding site, a heat shock element, and an E box [54]), it may still lack the critical *cis* element(s) responsible for the transcriptional activation of the endogenous *MKP-1* gene by these treatments. Future studies are required to explore these possibilities.

Role of the p38 pathway in arsenite-triggered histone H3 modification. We have shown that both the nucleosomal changes and the *MKP-1* gene induction in response to arsenite are partially mediated by p38. The fact that the MEK inhibitor U0126 had little effect on histone H3 modification is in good agreement with the relatively weaker and transient nature of ERK activation by arsenite (Fig. 2C) (16). Differences in histone H3 phosphorylation triggered by different stresses may be explained by the differential kinase activations induced by these treatments. It is worth noting that unlike other stresses, such as heat shock, UVC, or H_2O_2, arsenite caused a potent and sustained activation of p38 (Fig. 2D), which was likely due to the inhibitory effect of arsenite on MAP kinase phosphatases (12), including MKP-1 (data not shown). The sustained p38 activation correlated tightly with the activation kinetics of MSK1, which has been recently implicated in histone H3 phosphorylation (56) (Fig. 5). The overall similar kinetics of MSK1 activation and histone H3 phosphorylation suggests that histone modification in response to arsenite could be mediated, at least in part, by MSK1 (Fig. 4 and 5). This possibility is supported by the finding that both processes were inhibited by SB203580 but not U0126 (Fig. 5). It is important to note that, in addition to p38, other signals generated by arsenite may also contribute to histone H3 phosphorylation-acetylation. Such a notion is consistent with the observation that SB203580 did not completely abolish arsenite-triggered histone H3 modification

(Fig. 4A) while activation of MAPKAPK2/3, a direct target of p38, was virtually eliminated (data not shown). In addition to protein kinases regulated by the MAP kinase cascades (RSK2 and MSK1), cAMP-dependent protein kinase has also been implicated in histone H3 phosphorylation (61). Protein kinases responsible for histone H3 phosphorylation during mitosis were recently identified in lower eukaryotic organisms (26, 31, 59). It is possible that some of these kinases could be activated by arsenite and contribute to the histone H3 phosphorylation induced by arsenite.

Arsenite is a potent carcinogen that has been implicated in the development of skin and bladder cancers (3). We have previously demonstrated that arsenite can stimulate EGF receptor activity and induce the expression of c-*jun*, c-*fos*, and c-*myc* (16) (Fig. 7). ChIP assays indicated that arsenite also stimulates the phosphorylation-acetylation of histone H3 on the chromatin associated with the c-*fos* promoter (Fig. 7). Paradoxically, arsenite is also a very effective therapeutic agent for the treatment of a subset of acute promyelocytic leukemia (APL) associated with retinoic acid receptor gene translocation (36, 48). Interestingly, recent studies have suggested that failure to initiate histone acetylation at retinoic acid-regulated genes is responsible for the development of APL (36, 37). Furthermore, histone deacetylase inhibitors together with retinoic acid can stimulate the differentiation of the subset of APL that is insensitive to arsenite (36, 62). Our finding that arsenite induces histone H3 phosphorylation-acetylation may provide additional insight into the mechanisms for both the carcinogenic properties and the therapeutic effects of arsenite.

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